PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference L/2AR80/ES/57	**FOR FURTHER ACTION** See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP 03/13676	International filing date *(day/month/year)* 02.12.2003	Priority date *(day/month/year)* 03.12.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant BIOMERIEUX B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand 02.07.2004	Date of completion of this report 29.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Knehr, M Telephone No. +31 70 340-4277

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/13676

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-24 as originally filed

Sequence listings part of the description, Pages

1-6 received on 09.03.2004 with letter of 08.03.2004

Claims, Numbers

1-19 received on 19.01.2005 with letter of 17.01.2005

Drawings, Sheets

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 1-12 (part.)

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 1-12 (part.) are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☒ the claims, or said claims Nos. 1-12 (part.) are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 1-12 (part.)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/ or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9,11-19
	No:	Claims	10
Inventive step (IS)	Yes:	Claims	1-9,11-18
	No:	Claims	10,19
Industrial applicability (IA)	Yes:	Claims	1-19
	No:	Claims	

2. Citations and explanations

see separate sheet

I. Basis of opinion (Continuation)

I.1 Amended claims filed with letter from 17 January 2005 do not extent beyond the content of the application as originally filed, thus fulfilling the requirements of Article 41 PCT as well as Rule 66.8 PCT.

III. Non-establishment of opinion (Continuation)

III.1 As explained within the International Search Report, claims 1-12 insofar they do not relate to a (molecular beacon) probe comprising defined and supported nucleotide analogues and the use of such (molecular beacon) probes, were not subject of a search, and therefore are not subject to examination (Rule 66.1(e) PCT).

III.2 From the application as a whole, it appears to the International Preliminary Examination Authority (IPEA) that the core of what is claimed, deals only with molecular beacon probes (MB probes) and not with nucleic acid probes in general, due to the problem to be solved (see point 2.3.2). Any generalization in view of the probes, e.g. according to the Guidelines C-III-6.5, can only be granted, if the skilled reader could understand other possible examples imaginable within the context and the scope of the application. Applying the problem/solution approach to what is defined as the scope of the claims, it appears to the skilled person that nothing else than the presence of modified nucleotides within the 'stem' structure of a probe of the invention could be meant, and such a stem structure is inevitably linked to the use of molecular beacons as analytical probes. In addition, besides MBs, no other kind of probes are either disclosed nor supported within the description. Thus, the limitation of the search, as well as the need to reduce the possible kind of probes of the invention to MBs only, must be maintained.

III.3 Claims 1-12 do not meet the requirements of Articles 5 and 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt (in parts) to define the subject-matter in terms of results to be achieved which merely amounts to a statement of the problem(s) to be solved (see detailed reasoning given within the International Search [PCT/ISA/210]). The technical features necessary for achieving these results should not only be added, but also defined by clear terms having a basis within the application and being understandable for a person skilled in the art. The Guidelines C-III-4.7 allow such a definition

only when the claims cannot be defined otherwise more precisely without unduly restricting the scope of the claims. Related to product claims 10-19, as well as claims 1-9 making use of the products of clams 10-19, it is however, well possible to define the scope of the claims by clear technical features unambiguously characterizing the products of the invention as well as their use.

III.4 In addition, the present application does not meet the requirements of Article 6 PCT because of the excessive number of independent product claims (claims 10, 11 and 12, all relating to [molecular beacon] probes), as well as independent use claims (claims 1, 2, 4 and 5, relating to such MB probes within a diagnosing hybridization assay), giving rise to a lack of conciseness and clarity, since the plurality of independent claims of each category makes it difficult, if not impossible, to determine unambiguously the matter for which protection is sought, thus placing an undue burden on others seeking to establish the extent of protection (contrarily to the requirements of Article 6 PCT). Therefore, examination referring to novelty, inventive step and industrial applicability, was executed according to the limitations as described within the International Search (as defined under III.1 to III.3), i.e. a molecular beacon probe comprising nucleotide analogues selected from 2'-O-methyl nucleotides or LNA nucleotides, and the use of such a probe within a diagnostic hybridisation assay.

III.5 Finally, independent claims 1, 2, 4, 5 and 11, relate to products (molecular beacon probes) partially defined by methodological steps, i.e.: 'Use...of a probe/Molecular beacon probe for use...,**which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify and detecting the amplified analyte or its complement by means of a probe**, ...'. That bold parts of claims 1-9 and 11 do not at all contribute in characterizing the claimed probe by technical features. An attempt is made in defining the probes by steps within a non-related amplification method. Since no potential link exists between such a method and a molecular beacon probe, due to the nature of the latter being a 'detection mean', again a lack of clarity arises for claims 1, 2, 4, 5 and 11 (in contrast to Article 6 PCT).

V. Reasoned statement (Continuation)

2.1 CITATIONS

The following documents are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: TSOURKAS ET AL.: NUCLEIC ACIDS RESEARCH, vol.30(23), 1 December 2002, 'Hybridization of 2'-O-methyl and 2'-deoxy molecular beacons to RNA and DNA targets', pages 5168-5174.
D2: WO 0066604 (EXIQON) 9 November 2000 (2000-11-09)
D3: MAJLESSI ET AL.: NUCLEIC ACIDS RESEARCH, vol.26(9), 1998, 'Advantages of 2'-O-methyl oligoribonucleotide probes for detecting RNA targets', pages 2224-2229.
D4: WO 03020952 (GEN-PROBE INCORPORATED) 13 March 2003 (2003-03-13)

2.2 NOVELTY (Art. 33(2) PCT)

2.2.1 Document D4 published between the priority date and the filing date essentially disclose (parts of) the content of the application as filed. Under the current procedure according to the PCT, this document is irrelevant for evaluating aspects of novelty and inventive step. However, in case, the applicant will pursue the European procedure (EPC), the following has to be taken into account: Dependent of the outcome of checking the validity of priority (i.e. in case the priority would not be valid), D4 could be cited against novelty and inventive step of the (entire set of) claims.

2.2.2 D1 discloses molecular beacons probes (MBs) consisting from 2'-O-methyl substituted nucleotide analogues, making these MB probes less sensitive for degradation by nucleases, thus preventing non-desired background fluorescence following undesired opening of the MBs, and thus preventing the effect of undesired opening ('IBF effect'). D1 further discloses the suitability of such MBs for better discrimination of mismatches between probe and target sequences, therefore lowering the effects of sequence variations in hybridization assays (abstract; page 5168, col.2, paragraphs 2-3; page 5169, col.2, paragraph 2; as well as table 1). In view of D1, claim 10 is not novel since it does not exclude a molecular beacon probe comprising only 2'-O-methyl substituted nucleotide analogues.

2.2.3 Likewise, document D3 discloses advantages of detection probes comprising

2'-O-methyl oligoribonucleotide analogues for the detection of RNA targets. D3 specifically mentions their superiority over 2'-deoxy ribonucleotide probes in regards of RNA target affinity, increased Tm's, faster hybridization kinetics, suitability as probes within diagnostic hybridisation assays, as well as the significantly improved discrimination between wild-type and mismatches RNA targets (the whole document). Also in view of D3, claim 10 is not novel over the prior art.

2.2.4 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claim 10 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.2.5 However, the subject-matter of claims 1-9 and 11-19, can be considered to be new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 Document D1 is considered to represent the most relevant state of the art and discloses molecular beacons probes (MBs) consisting from 2'-O-methyl substituted nucleotide analogues, and the use of such probes for hybridisation assays, due to their suitability for...
a) ...preventing non-desired background fluorescence following undesired opening of the MBs, thus preventing the so-called 'IBF effect', and
b) ...better discrimination of mismatches between probe and target sequences, therefore lowering the effects of sequence variations in hybridization assays.
The subject-matter of independent product claims 11 and 12 differs in that D1 discloses MBs only consisting of continuous stretches of 2'-O-methyl substituted nucleotide analogues, possessing no unmodified nucleotides, neither in the stem nor in the loop of the MBs. The effect of that difference, i.e. MBs including partially unmodified nucleotides, lies in a further reduction of background fluorescence, as well as a further improvement in discriminating between sequence variants, due to mismatches.
The subject-matter of independent claims 1, 2, 4 and 5, makes use of such MBs, relying on the effect of their structural difference with the prior art (as reflected by D1).

2.3.2 The problem to be solved by the subject matter of claims 11, 12, as well as 1, 2, 4 and 5, can therefore be defined as the need for means for achieving these effects. The solution are MB probes possessing at least one 2'-O-methyl- or LNA-substituted nucleotide analogue, but more important discontinuous stretches of such analogues comprising also unmodified nucleotides, especially within the stem of such an MB probe.

2.3.3 Besides using 2'-O-methyl substituted nucleotide analogues within hybridization probes, the prior art also teaches the incorporation of locked nucleotide analogues (LNAs) as well as amplification and diagnostic methods making use of such probes. D2 further discloses enhanced affinity properties within hybridization methods using such LNA-comprising probes (abstract; page 44, last paragraph - page 45, paragraph 3; page 47, paragraphs 2-4; page 48, paragraph 3 - page 49, last paragraph; examples 10 and 11, Fig.'s 2 and 3; as well as the claims).

2.3.4 In view of the prior art reflected either by D1 or D2, at first sight, it appears that the principle of making use of MB probes comprising either 2'-O-methyl substituted nucleotide analogues or LNAs, especially for the very same purpose of preventing non-desired opening of the MBs, as well as better discrimination of mismatches between probe and target sequences, therefore lowering the effects of sequence variations in hybridization assays, is perfectly known from the prior art, and therefore, cannot be inventive.

2.3.5 However, it is a surprising effect that reduction in number of modified oligonucleotides gives rise to improved mismatch discrimination as well as prevention of premature MB probe opening. Therefore, the IPEA is of the opinion that it would not be obvious for a person skilled in the art when faced with the above mentioned problem, to reduce the number of nucleotide analogues within a MB hybridisation probe. Instead, it could have been expected from the prior art that the incorporation of further nucleotide analogues would lead to such an effect. Since nothing within the prior art points to that solution, it therefore appears that independent claims 1, 2, 4, 5, 11 and 12, do comprise an inventive step (Art. 33(3) PCT).

2.3.6 The same is valid for dependent claims 3, 6-9, and 13-18.

2.3.7 The kit of claim 19, suitable for performing a diagnostic amplification assay, referring (in part) to claim 10, is not considered inventive. Since the probe of claim 10 is

regarded as being not novel, in can neither be inventive. And therefore, the packaging of non-inventive subject-matter into a kit would be obvious to the skilled person. Thus, claim 19 does not satisfy the criterion set forth in Article 33(3) PCT, and its subject-matter does not involve an inventive step (Rule 65(1)(2) PCT).

2.3.8 The present application does not satisfy the criterion set forth in Article 33(3) PCT since the subject-matter of claims 10 and 19, does not involve an inventive step as set forth in Rule 65(1)(2) PCT.

2.3.9 However, the present application does satisfy the criterion set forth in Article 33(3) PCT since the subject-matter of claims 1-9, and 11-18, does involve an inventive step as set forth in Rule 65(1)(2) PCT.

EPO - DG 1
19. 01. 2005
(71)

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enclosure to letter dated 17-01-2005

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AMENDED CLAIMS

1. Use in a diagnostic hybridization assay of a probe for lowering the effect of sequence variations in a nucleic acid analyte, which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, characterized in that the probe comprises:

- one or more nucleotides and/or nucleotide analogues, selected from 2'-O-methyl nucleotides or LNA nucleotides, that have an affinity increasing modification and the diagnostic assay is for assessing the amount of analyte present in the sample, and
- one or more unmodified nucleotides.

2. Use in a diagnostic hybridization assay of a probe for lowering the effect of sequence variations in a nucleic acid analyte, which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, characterized in that the probe comprises:

- one or more nucleotides and/or nucleotide analogues, selected from 2'-O-methyl nucleotides or LNA nucleotides, that have an affinity increasing modification, i.e. at a constant temperature of hybridization, the melting temperature of the probe with any possible analyte's polymorphism is increased compared to the melting temperature of an unmodified probe with any analyte's

polymorphism and the diagnostic assay is for assessing the presence of the analyte in the sample

- one or more unmodified nucleotides.

3. Use as claimed in claims 1-2, wherein the probe is a molecular beacon.

4. Use in a diagnostic hybridization assay of a molecular beacon probe for lowering the possible opening of the stem of the molecular beacons by way of at least one contaminant present in the amplification enzymes mixture, which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, characterized in that the probe's stem comprises:

- one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, especially 2'-O-methyl nucleotides, and
- one or more unmodified nucleotides.

5. Use in a diagnostic hybridization assay of a molecular beacon probe for lowering:

- the effect of sequence variations in a nucleic acid analyte, and/or
- the possible opening of the stem-loop structure of the molecular beacons by way of at least one contaminant present in the amplification enzymes mixture,

which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to

amplify the analyte and detecting the amplified analyte or its complement by means of the probe, characterized in that the probe's loop comprises:

- one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and
- one or more unmodified nucleotides.

and/or the probe's stem comprises:

- one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, especially 2'-O-methyl nucleotides, and
- one or more unmodified nucleotides.

6. Use as claimed in any one of the claims 1-5 wherein the diagnostic assay is a homogeneous assay.

7. Use as claimed in any one of the claim 1-5 wherein the diagnostic assay is a heterogeneous assay.

8. Use as claimed in any one of the claims 1-7, wherein the nucleotides or nucleotide analogues having an affinity increasing modification are selected from the group consisting of 2'-O-derivatized nucleotides, locked nucleic acids and peptide nucleic acids.

9. Use as claimed in claim 8, wherein the 2'-O-derivatized nucleotide is a 2'-O-methyl-nucleotide.

10. Molecular beacon probe for use in a diagnostic hybridization assay, said probe comprises one or more nucleotides and/or nucleotide analogues, selected from 2'-O-methyl nucleotides, that have an affinity increasing

modification i.e. at a constant temperature of hybridization, the melting temperature of the probe with any possible analyte's polymorphism is increased compared to the melting temperature of an unmodified probe with the same target.

11. Molecular beacon probe for use in a diagnostic hybridization assay, said probe allowing the lowering of the possible opening of the stem-loop structure of the molecular beacons by way of at least one contaminant present in the amplification enzymes mixture, which assay comprises the steps of contacting a set of primers and a sample containing the nucleic acid analyte to amplify the analyte and detecting the amplified analyte or its complement by means of the probe, characterized in that the probe's stem comprises:

- one or more 2'-O-methyl nucleotides, and
- one or more unmodified nucleotides.

12. Molecular beacon probe for use in a diagnostic hybridization assay, said probe allowing the lowering of:

- the effect of sequence variations in a nucleic acid analyte, and/or
- the possible opening of the stem-loop structure of the molecular beacons by way of enzymes, characterized in that the probe's loop comprises:
- one or more nucleotides and/or nucleotide analogues that have an affinity increasing modification, and
- one or more unmodified nucleotides.

and/or the probe's stem comprises:

- one or more 2'-O-methyl nucleotides, and
- one or more unmodified nucleotides.

13. Probe or molecular beacon probe as claimed in any one of the claims 11-12, wherein the nucleotides or nucleotide analogues having an affinity increasing modification are selected from the group consisting of 2'-O-derivatized nucleotides, locked nucleic acids, peptide nucleic acids.

14. Probe or molecular beacon probe as claimed in claim 13, wherein the 2'-O-derivatized nucleotide is a 2'-O-methyl-nucleotide.

15. Molecular beacon probe as claimed in any one of the claims 11-14, wherein each base pair constituting the stem contains no more than one 2'-O-methyl nucleotide.

16. Molecular beacon probe as claimed in any one of the claims 11-15, wherein at least one base pair constituting the stem contains no nucleotide or nucleotide analogue having an affinity increasing modification.

17. Molecular beacon probe as claimed in any one of the claims 11-16, wherein one base pair constituting the stem contains no nucleotide or nucleotide analogue having an affinity increasing modification

18. Molecular beacon probe as claimed in any one of the claims 11-17, wherein each strand constituting the stem contains at least one nucleotide or nucleotide analogue having an affinity increasing modification.

19. Kit for performing a diagnostic amplification assay, comprising the appropriate primers, polymerase(s) and reagents for performing amplification of an analyte to be

diagnosed and a probe or a molecular probe as claimed in claims 10-18 for detecting the amplified analyte.